ENVIROMENTAL LIVER TUMORS

HANS POPPER AND LOUIS B. THOMAS

The Stratton Laboratory for the Study of Liver Disease, The Mount Sinai School of Medicine, New York, New York and the Laboratory of Pathology, National Cancer Institute, NIH, Bethesda, Maryland 20014, U.S.A.

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The recent realization that the majority of human carcinomas are induced by chemical agents has raised concern all over the world as to the possible induction of these tumors by environmental factors, in part derived from industrial sources. Both industrial production of chemicals as well as their pollution of the environment have markedly increased and present a hazard not only for workers in contact with carcinogenic agents, but for the population at large exposed to ambient air and food. Storage of compounds such as organochloride pesticides and polychlorinated biphenyls in fat tissue of man has been demonstrated, which enhances the possibility of hidden sources of human carcinogenesis. Indeed, since the initial demonstration of scrotum carcinoma in chimney sweeps by Pott in 1775, many examples of environmental malignancies have been demonstrated in man, such as mesotheliomas and lung carcinomas from exposure to asbestos, lung carcinomas from smoking, leukemias and lymphomas from radiation. Epidemiologic and geographic pathologic studies, many also conducted in Japan, have raised the serious possibility that carcinomas of the gastrointestinal tract, particularly of the stomach and colon and also of the pancreas, are of environmental etiology (Higginson, 1976). The importance of these considerations cannot be overemphasized since the elimination of these hazards would prevent this scourge of humanity.

Significance and incidence of environmental hepatic tumors

The pioneering work of Kinoshita and Yoshida (Yoshida, 1932) in producing hepatocellular carcinomas in experimental animals by azo-compounds in 1932 raised the possibility that hepatic tumors in man are of chemical and thus of potential environmental origin. Indeed a large number of chemicals have been shown to produce hepatocellular carcinoma and also angiosarcoma in experimental animals (Farber, 1976), particularly in rodents but also in subhuman primates, with the N-nitroso-compounds producing both. In primates (Adamson, 1972), both types of hepatic tumors are often found in the same animal. The liver appears a priori a preferred site of carcinogenesis because many of the agents, being lipid-soluble, are transformed into the nucleophilic or ultimate carcinogen, primarily in its microsomal biotransformation system, located in the smooth endoplasmic reticulum.

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189
This bioactive metabolite has the potential of binding with cellular macromolecules, of which DNA appears at this time to be the crucial component since its alterations set in motion a carcinogenic chain (Popper, in press a). It is also reflected in the mutagenic potential demonstrated, for instance, by the Ames test in bacteria.

However, additional factors in this carcinogenic chain determine the development of carcinoma. The first is the amount and life span of the bioactive compound, usually a hydroxylate, an epoxide or an alkalizing agent. As a rule, it undergoes further, mainly enzymatic, biotransformation which includes hydration, binding to glutathione, or glucuronic acid conjugation. These processes result in abolition of the carcinogenic potential and may render the agent water-soluble for excretion by the cell and even by the body. Both the multistep formation and degradation of the bioactive agent are influenced by a large number of other factors which include drugs, environmental compounds such as pesticides and polychlorinated biphenyls, smoking and alcohol. Also, genetic, hormonal, nutritional, climatic, and even psychologic elements play a role, as does the blood flow. Thus, a series of processes, in fact unpredictable in the individual person, determine the availability of the agent capable of covalent binding to macromolecules (Remmer et al., in press).

The second factor is the possibility of removal of the altered portion of DNA by repair enzymes of endonuclease character. Their activity thus determines the carcinogenic potential.

A third factor in the carcinogenic chain is the fixation of the DNA alteration in that hepatocytes in division, that means in the s phase, appear more susceptible to binding and in that proliferation of cells not only renders them more susceptible but also fixes the alteration in the template.

The described events of initiation occur during a relatively short period, almost like a flash. But hepatocytes thus altered have to undergo a series of divisions to develop into carcinoma. This sequential promotion requires a long time in man, usually years. The morphologic and biochemical changes characteristic of precarcinomatous hepatocytes have been well studied, particularly by Farber (Solt et al. 1977) and Pitot (Pitot, 1977). These hepatocytes have reduced susceptibility to toxic agents and show loss of adult enzymes such as glucose-6-phosphatase or 5-nucleotidase, but contain embryonal enzymes such as gamma-glutamyl transpeptidase or oncofetal proteins (alpha-fetoprotein or carcino-embryonic antigen), and in man, excess alpha-1-antitrypsin.

Environmental agents may thus act in two ways. One is the modulation of the carcinogenic chain either by increasing the availability of the bioactive metabolite of another compound or by stimulating hepatocellular proliferation. The other is, to serve as the ultimate carcinogen or, more often, as its precursor. Experimental observations on many environmental chemicals offer ample examples of the first possibility, particularly as to induction of the enzymes in the biotransformation system, both forming and degrading. This includes many of the agents which by themselves need not have a potential for altering DNA, as reflected, for instance, in a lack of mutagenic capacity, even after activation by hepatic microsomal systems. In man, however, the extent of such induction as well as its consequences for carcinogenesis are so far not established, but convincing evidence exists for adverse
drug reactions in man by a combination of inducing drugs with others transformed in the system as exemplified in antituberculous therapy with rifampicin and isoniazid (Pessayre et al., 1977). Nevertheless, epidemiologic studies failed to detect an increase of hepatic malignancies in epileptics after long-term administration of inducing drugs. The problem of induction in man by environmental agents in producing hepatic tumors deserves extensive additional studies.

By contrast, surprisingly little evidence exists that the common human carcinogens with strongly implicated potency in other organ systems, namely, polycyclic hydrocarbons and N-nitroso-compounds, produce malignancies in the human liver, despite their accumulation and metabolism in this organ. Polycyclic hydrocarbons do not induce tumors in any species and there is so far no proof for human hepatic tumors produced by N-nitroso-compounds which readily induce them in experimental animals. This protection of the human liver, at least in the adult, might be explained by the high hepatic activities of hydratase (Walker et al., 1978) and of endonucleases as well as by the long life span and with it, slow turnover of the human hepatocyte. Thus for man the number of established or even strongly suspected hepatic carcinogens is restricted to a relative small group of agent. Also, a search of the literature revealed otherwise mostly transient hepatic environmental injuries and only occasional acute fatal necrosis after accidental overdose. The evidence for human cirrhosis from environmental chemicals other than those listed below is not convincing since it is also restricted to accidental heavy overexposure. There is adequate epidemiological evidence that aflatoxin, the most potent carcinogen by unit weight, and possibly other mycotoxins produce hepatocellular carcinoma in man (Peers et al., 1976; Wogan, 1976) and the metabolic basis has been well worked out (Roebuck et al., 1978). Alcoholism is related to hepatocellular carcinoma, almost always associated with preexisting cirrhosis (Martini, in press), but the pathogenesis is not established. It is the most frequent association where the carrier rate of hepatitis B is low. Since it supposedly is more frequent in re-formed alcoholics (Lee, 1977), longer survival may explain the recent frequency. Cirrhotic processes may favor abnormal regenerative processes demonstrable histologically or predispose for the action of other ubiquitous carcinogens (Popper, 1977a).

The third factor is hepatitis B and possibly also hepatitis not-A not-B. In the former the increased risk of the carrier stage is well substantiated (Kew, in press). The mechanism of the carcinogenesis in hepatitis B-infected persons is not settled and a linkage of chemical and viral carcinogenesis is an attractive hypothesis. Aflatoxin has been incriminated here. The rarer association of hepatocellular carcinoma with hemochromatosis, alpha-1-antitrypsin deficiency and tyrosinosis have no apparent environmental etiology.

The best studied pathologic sequences leading to environmental hepatic tumors in man (all without cirrhosis) result from the effects of anabolic and possibly also of contraceptive steroids and the one induced by vinyl chloride, which latter is identical with the lesions associated with medicinal or environmental exposure to arsenic and following administration of Thorotrast, although not all the listed agents can be considered environmental in the strict sense of the term (Popper et al., 1977b). They are a hazard to only a
small segment of the population. Their study, however, provides useful information not only for environmental pathology but for general pathobiology as well as for the extrapolation of animal experimental studies in man.

Steroid-induced hepatic tumors and environmental considerations

Hepatic adenomas are observed, more often in the right lobe and sometimes multiple, in men and women receiving large doses of anabolic steroids, primarily for treatment of aplastic anemia, and in very few women taking contraceptive pills as well as following pregnancy but occasionally also without established relation (Klatskin, 1977). The adenomas consist of hepatocytes differing from the surrounding parenchyma by high glycogen content and increased size, but their appearance is usually uniform (Edmondson et al., 1976). The lobular architecture is missing or distorted, the portal structures may fail to reveal bile ducts, and excess of isolated veins and arteries is frequent. The blood supply is predominantly arterial. Dilated arteries, sometimes on the edge, permit arteriographic recognition not always possible by scanning. The sinusoids are often irregularly dilated and this may progress to bloody cysts (peliosis). Moreover, in the adenomas associated with the intake of contraceptive drugs, acid mucopolysaccharides accumulate in the intima of arteries and veins, sometimes obstructing the lumen (Irey et al., 1970). These vascular changes predispose to intrahepatic bleeding which may progress to peritoneal hemorrhage. The sinusoidal dilatation may be the most important cause. It has also been described in women on contraceptive drugs without tumor formation (Winkler and Poulsen, 1975) and in men and women on anabolic steroid therapy; peliosis may even cause death, in part explained by hepatic failure (Bagheri and Boyer, 1974). The regressive changes in adenoma induce fibrosis and this sometimes raises problems in differentiation from focal nodular hyperplasia. This lesion occurs, sometimes as an incidental finding, in any age group and in both sexes, although more frequently in females. However, even morphologically typical focal nodular hyperplasias with a central fibrotic core and septa radiating into the surrounding parenchyma were sources of bleeding in women on contraceptive medication, possibly explained by an action of steroids on a preexisting hamartoma.

In addition to their clinical interest, the hepatic adenomas are important in extrapolation to man of morphologically similar tumors in experimental chemicals, but also drugs and especially sex steroids, induce in rodents hepatic nodules designated as hyperplastic or neoplastic which show features identical to the ones found in human adenomas. They proceed from hyperplastic areas to well defined nodules, often deforming the liver (Squire and Levitt, 1975). Variations in cell populations, designated as “nodules in nodules” (Popper et al., 1960), are transitions to hepatocellular carcinoma. These rodent nodules are considered an indication of neoplastic potential and have thus served as the basis for banning of compounds by regulatory agencies. The recent recognition of hepatic adenomas in man supports this conclusion although species differences in hepatic, primarily microsomal, biotransformation deserve serious consideration.

The similarity of rodent nodules to steroid-related hepatic adenomas in man includes development of “nodules in nodules”. However, so far, progression to carcinoma of human adenomas
associated with use of contraceptive drugs has not been observed and, indeed, regression on discontinuation of steroid therapy has been described, at least on the basis of scanning. However, carcinomas in the center of adenomas have been found in such women (Davis et al., 1975), and carcinomas in otherwise normal livers have been described in women on contraceptive drugs. However, since such carcinomas occur in this age group without known cause, although in low frequency, a causal relation is today not proven. However, there is far less doubt as to the progression of the lesion induced by anabolic steroid therapy to carcinoma on the basis of the frequency of carcinomas in the relatively few patients on large doses of these drugs.

The causal relation between contraceptive drugs and hepatic adenomas is clouded by their low incidence if one considers that not more than 400 such adenomas have been reported, mainly from the United States, England and Scandinavia, when 30 Million women are taking such drugs at one time in the United States alone. In the mentioned countries, use of contraceptive steroids has been widespread for ten years while in the rest of Europe such use is more recent. However, the reported increased incidence, at least in Germany, in the last two years (Altman, in press) suggests a causal relation, although in extremely low incidence. The clinical significance of these tumors, which seem not to be associated with conspicuous rise of alpha-fetoprotein, is the possibility that an intraperitoneal hemorrhage in a woman of reproductive age may not only be the result of a tubal rupture but can also be caused by a bleeding hepatic adenoma, requiring partial hepatectomy which demands more skillful surgical intervention.

The sequence exemplified by exposure to vinyl chloride

At least three agents, namely vinyl chloride, arsenic and Thorotrast, are associated with a characteristic morphologic and clinical sequence (Popper et al., in press b). The morphologic features have been fully reproduced in rodents, primarily by the experiments of Maltoni in Bologna (Maltoni and Lefemine, 1974; Popper et al., 1977b). This represents probably the best documentation of similar precarcinogenic hepatic sequences in man and animals and underscores the importance of the experimental model. Indeed, Maltoni's production of hepatic angiosarcoma in rodents by inhalation of vinyl chloride (Maltoni and Lefemine, 1974) proceeded the recognition of similar tumors in workers exposed to gaseous vinyl chloride, primarily in the polymerization of the monomer to polyvinyl chloride (Creech and Johnson, 1974). Although Maltoni's studies were initially not fully appreciated, they subsequently served in establishing acceptable levels of vinyl chloride in the ambient air in factories.

In man the initial lesion is a focal hyperplasia and hypertrophy of hepatocytes which vary markedly in appearance (Fig. 1). Subsequently it is combined with a proliferation of various sinusoidal lining cells, including endothelial cells, PAS-positive macrophages, fat-storing or Ito cells, as well as lymphocytes and a few segmented leukocytes. This sinusoidal cell proliferation is associated with a focal increase of the reticulum. Silver impregnation is the most useful histological method to recognize this precursor stage (Fig. 2A). Electron microscopy shows a characteristic pericellular fibrosis, as well as an increase of Ito cells (Triche et al., 1975; Kurokawa et al., 1975). A non specific portal fibrosis
is also frequent, as is a laparoscopic picture of an irregular nodular thickening of the hepatic capsule as was well demonstrated by German investigators in vinyl chloride workers (Marsteller et al., 1973; Marsteller et al., 1975). In this stage, portal hypertension may be noted clinically and esophageal varices with hemorrhage are its early manifestation. The spleen is often conspicuously enlarged and shows enlarged Malphigian follicles in which recent hemorrhage and calcium and iron inclusions (Gamma-Gandy bodies) may be seen. In short, the features designated conventionally as idiopathic portal hypertension or Banti’s syndrome occur in vinyl chloride-exposed workers, raising the possibility that other instances of idiopathic portal hypertension, rare in the Western world, may also have a toxic etiology (Popper, 1977a).

The subsequent evolution of the human lesion towards angiosarcoma follows essentially two pathways (Popper et al., in press b). In the less frequent one the proliferation of sinusoidal cells progresses (Fig. 2B) in that endothelial cells with bizarre nuclei predominate and pile up to occasionally obstruct the sinusoidal lumen and to produce the characteristic intralobular angiosarcoma in which the hepatic cords are eventually replaced by fibrous tissue. The same pathway is observed in rodents in which the initial lesion is also a focal, usually nodular, hyperplasia and hypertrophy of hepatocytes (Fig. 3A) which electron-microscopically reveal excess of the smooth endoplasmic reticulum and alteration of the plasma membrane in the form of invaginations (Popper et al., 1977c). Again, various sinusoidal cells proliferate in the nodule, and in silver impregnations the reticulum framework is increased (Fig. 3 B). This contrasts to the previously described neoplastic nodules of rodents produced by a large number of environmental agents in which the reticulum framework is decreased. In rats exposed to vinyl chloride shortly after birth, progression of these nodules to hepatocellular carcinoma has been observed by Maltoni (Fig. 4 A) (Maltoni, 1976), while in older rats, angiosarcoma, which may be of the intralobular variety, is more frequent (Fig. 4 B).

The second pathway to angiosarcoma, more frequent in man, starts with a conspicuous dilatation of the sinusoids, apparently first in the hyperplastic nodules. The widening of the sinusoids loosens the lobular architecture in that trabeculae consisting of hyperplastic hepatocytes, often in two-cell-thick cords, are covered by proliferated endothelial cells progressing to angiosarcoma (Fig. 5). The Disse spaces are widened and contain initially a variety of mesenchymal cells and abundant reticulum (Fig. 6). Eventually a trabecular angiosarcoma, the histologically most characteristic form, develops with widened blood spaces. Extramedullary hematopoietic foci are frequently seen in these sarcoma-lined spaces. Progressing fibrosis of the hepatocytic trabeculae leads to disappearance of the hepatocytes so that papillary structures with hyalinized fibrotic stalks are covered by angiosarcoma cells. Eventually, large bloody cysts are produced which are traversed by beam-like hyalinized remnants of the original portal tracts and central canals. In both trabecular and intralobular angiosarcoma nodular aggregates of sarcoma cells may develop which are devoid of inflammatory cells except where there is secondary ischemic necrosis. These nodular aggregates consist of either spindle-shaped cells (Fig. 6 B), in part lining vascular
spaces, or of polyhedral cells which because of their bulky cytoplasm appear epithelioid. Again, in rodents sinusoidal dilatation is noted (Fig. 7A), and on electron microscopy, gaps are observed in the endothelial lining which are covered by an accumulation of platelets, supporting a specific sinusoidocidal action. A proliferation of mesenchymal cells is followed by eventual prominence of tumor cells which have the cytologic characteristics of endothelial cells (Popper et al., 1977c). Also, in rodents loosening of the lobular architecture separates cords of proliferated hepatocytes lined by angiosarcoma cells with finally complete destruction of the architecture, but with persistence of hyalinized fibrotic beams and accumulation of mainly polyhedral angiosarcoma cells without other inflammatory cells (Fig. 7B).

Both the nodular precursor lesion with mixed hepatocytic and sinusoidal cell hyperplasia associated with portal hypertension (Morris et al., 1974) and the mainly trabecular angiosarcoma (Regelson et al., 1968) have been observed in relatively few patients treated with large doses of arsenic in the form of Fowler's solution for psoriasis or asthma. This confirms previous experiences in Germany (Roth, 1957), where cirrhosis and cancer and particularly angiosarcoma have developed in vineyard workers spraying arsenic-containing pesticides. In addition, idiopathic portal hypertension has been observed in India, associated with high arsenic content in drinking water (Datta, 1976) and a case of hepatic angiosarcoma in association with well water contaminated with arsenic has also been reported (Rennke et al., 1971). Moreover, some instances of cryptogenic hepatic angiosarcoma studied by us are suggestive of environmental exposure to arsenic from the occupation of the patient. Epidemiologic studies are being undertaken to confirm the suspicion that environmental arsenical exposure is a possible cause of a significant number of cases of hepatic angiosarcoma.

In a series of hepatic angiosarcomas associated with exposure to Thorotrast, the same histologic sequence has been observed starting with focal mixed nodular hyperplasia and peliosis proceeding to angiosarcoma, modified only by the deposition of Thorotrast (Falk et al., in press). Although Thorotrast is not present in the sarcoma cells themselves, the lesion starts close to fibrotic foci around Thorotrast deposits.

Finally, the same sequence has been described in Portuguese vineyard workers exposed to a copper-containing pesticide (Pimentel and Menezes, 1977).

To summarize, several agents-vinyl chloride, arsenic, Thorotrast and possibly also copper-elicited in man a sequence of hepatic changes starting with mixed nodular hyperplasia and progressing to angiosarcoma and even occasionally to hepatocellular carcinoma. The latter has been observed following arsenic and Thorotrast exposure and, in a very few instances (Gokel et al., 1976), also observed following vinyl chloride exposure. The entire sequence of changes has been reproduced in experimental animals.

**CONCLUSIONS**

The presented observations indicate that environmental factors are a substantial cause of hepatic tumors in only a limited number of instances. Their eradication is difficult where change of life styles or habits would have to be made, as in alcoholism and perhaps with mycotoxins. Here the
relation between hepatitis B and environmental factors which are not fully understood presents a particular problem. Prevention is easier in the relatively small group where industrial or medicinal exposure is incriminated. But, the study of these small groups provided rewarding information as to questions not only of environmental but also of general pathological significance.

1. The observations obtained may be helpful in providing histologic parameters for screening for other industrial injuries since vinyl chloride probably shares its potential with other chemical, possibly industrial agents. Unfortunately, clinical examinations, including liver function tests, have been of limited usefulness in screening populations of industrial workers exposed to these chemicals.

2. The application of the rodent model for man has been confirmed by the similarity to the steroid tumors and by the identity of sequences following vinyl chloride exposure.

3. The importance of the variations in cell populations, the "nodules in nodules", in carcinogenesis has been illustrated.

4. The simultaneous proliferation of hepatocytes and sinusoidal, particularly the endothelial, cells accounts for the alternative development of carcinoma and angiosarcoma, depending possibly on variations in microsomal biotransformation suggested by carcinomas following vinyl chloride exposure of newborns and angiosarcomas of older animals. Moreover, the disappearance of the inflammatory reaction preceding angiosarcoma growth suggests a restraining influence of the inflammatory reaction in angiosarcoma.

5. The close relation of lipocytes (fat-storing Ito cells) to fibrosis and especially to reticulin formation supports their role in fibrogenesis (Kent et al., 1976).

6. Finally, the possibility has been raised that idiopathic portal hypertension may result from toxic environmental agents.

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ENVIRONMENTAL LIVER TUMORS

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**Fig. 1.** Worker exposed to vinyl chloride. Multiple nodular areas with proliferation of hepatocytes and sinusoidal lining cells (arrows). H&E, 40×

**Fig. 2 A**

**Fig. 2.** Worker exposed to vinyl chloride. (A) Mixed hyperplastic nodules with increased reticulin fibers around proliferated hepatocytes. Silver impregnation. 250×.
Fig. 2 B

Fig. 2. Worker exposed to vinyl chloride. (B) Intralobular angiosarcoma still associated with inflammatory reaction. H&E, 250×.

Fig. 3 A

Fig. 3. (A) Rat exposed to 500 PPM vinyl chloride. Nodules with hyperplasia of hepatocytes and sinusoidal lining cells. H&E, 100×.
ENVIRONMENTAL LIVER TUMORS

*Fig. 3 B*

Mouse exposed to 6000 PPM vinyl chloride. Focal increase of reticulin fibers in part around dilated sinusoids. Silver impregnation. 40.

*Fig. 4 A*

Rat exposed as a newborn to 500 PPM vinyl chloride. Hepatocellular arcinoma. H & E. 100×.
Fig. 4 B

Fig. 4. (B) Adult rat exposed to 30,000 PPM vinyl chloride. Intralobular angiosarcoma. Note anaplastic sinusoidal lining cells. H & E. 100×.

Fig. 5

Fig. 5. Worker exposed to vinyl chloride. Loosening of lobular architecture with angiosarcoma cells covering hepatocytes. Hematopoietic foci in dilated spaces. H & E. 50×.
Fig. 6. Worker exposed to vinyl chloride. (A) Trabecular angiosarcoma. Note cords of hyperplastic hepatocytes lined by angiosarcoma cells with widened Disse spaces containing a variety of cells. H & E. 250×. (B) Higher magnification of nodular angiosarcoma with spindle-shaped cells, in part lining vascular spaces. H & E. 400×.
Fig. 7. Rat exposed to 500 PPM vinyl chloride. H & E, 100X. (A) Peliotic dilatation of sinusoids with activation of sinusoidal lining cells and hematopoietic foci in their lumen (arrow). Hyperplasia of hepatocytes. (B) Trabecular angiosarcoma with polyhedral cells and persisting hyalinized remnants of portal tracts.